AD-773 521

THE OBTAINING OF BACTERIAL ALLERGENS BY THE ALKALINE-EXTRACTION METHOD AND THE INVESTIGATING OF THEIR PHYSICO-CHEMICAL PROPERTIES

V. F. Runova, et al

Foreign Technology Division
Wright-Patterson Air Force Base, Ohio

7 January 1974

Unclassified

Security Classification

AD 773521

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY *(Corporate author)*
Foreign Technology Division
Air Force Systems Command
U. S. Air Force

2a. REPORT SECURITY CLASSIFICATION
Unclassified

2b. GROUP

3. REPORT TITLE
THE OBTAINING OF BACTERIAL ALLERGENS BY THE ALKALINE-EXTRACTION METHOD AND THE INVESTIGATING OF THEIR PHYSICO-CHEMICAL PROPERTIES

4. DESCRIPTIVE NOTES *(Type of report and inclusive dates)*
Translations

5. AUTHOR(S) *(First name, middle initial, last name)*
V. F. Runova, V. Yu. Gavrilenkova

6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
1970	10	10

8a. CONTRACT OR GRANT NO.	9a. ORIGINATOR'S REPORT NUMBER(S)
b. PROJECT NO. JDX4	FTD-HT-23-403-74
c.	9b. OTHER REPORT NO(S) *(Any other numbers that may be assigned this report)*
d.	

10. DISTRIBUTION STATEMENT
Approved for public release; distribution unlimited.

11. SUPPLEMENTARY NOTES	12. SPONSORING MILITARY ACTIVITY
	Foreign Technology Division Wright-Patterson AFB, Ohio

13. ABSTRACT
06

DD FORM 1 NOV 68 1473

Unclassified

Security Classification

EDITED TRANSLATION

FTD-HT-23-403-74 7 January 1974
AP1044456

THE OBTAINING OF BACTERIAL ALLERGENS BY THE ALKALINE-EXTRACTION METHOD AND THE INVESTIGATING OF THEIR PHYSICO-CHEMICAL PROPERTIES

By: V. F. Runova, V. Yu. Gavrilenkova

English pages: 10

Source: Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii, Nr. 10, 1970, pp72-77

Country of Origin: USSR
Translated by: Joseph E. Pearson
Requester: FTD/PDTR/D. G. Matheson
Approved for public release; distribution unlimited.

U. S. BOARD ON GEOGRAPHIC NAMES TRANSLITERATION SYSTEM

Block	Italic	Transliteration	Block	Italic	Transliteration
А а	*А а*	A, a	Р р	*Р р*	R, r
Б б	*Б б*	B, b	С с	*С с*	S, s
В в	*В в*	V, v	Т т	*Т т*	T, t
Г г	*Г г*	G, g	У у	*У у*	U, u
Д д	*Д д*	D, d	Ф ф	*Ф ф*	F, f
Е е	*Е е*	Ye, ye; E, e*	Х х	*Х х*	Kh, kh
Ж ж	*Ж ж*	Zh, zh	Ц ц	*Ц ц*	Ts, ts
З з	*З з*	Z, z	Ч ч	*Ч ч*	Ch, ch
И и	*И и*	I, i	Ш ш	*Ш ш*	Sh, sh
Й й	*Й й*	Y, y	Щ щ	*Щ щ*	Shch, shch
К к	*К к*	K, k	Ъ ъ	*Ъ ъ*	"
Л л	*Л л*	L, l	Ы ы	*Ы ы*	Y, y
М м	*М м*	M, m	Ь ь	*Ь ь*	'
Н н	*Н н*	N, n	Э э	*Э э*	E, e
О о	*О о*	O, o	Ю ю	*Ю ю*	Yu, yu
П п	*П п*	P, p	Я я	*Я я*	Ya, ya

* ye initially, after vowels, and after ъ, ь; e elsewhere. When written as ё in Russian, transliterate as yё or ё. The use of diacritical marks is preferred, but such marks may be omitted when expediency dictates.

THE OBTAINING OF BACTERIAL ALLERGENS BY THE ALKALINE-EXTRACTION METHOD AND THE INVESTIGATING OF THEIR PHYSICO-CHEMICAL PROPERTIES

V. F. Runova and V. Yu. Gavrilenkova,
Tarasevich Control Institute of Medical
and Biological Preparations
(Signed to press 9 Dec. 1969)

A common method of obtaining allergens from bacteria of the group of particularly dangerous infections and of certain representatives of the enteric family is suggested as a result of the work carried out. The method consists in the extraction from microbial cells of alkali-soluble components, their precipitation with acid, dialysis and lyophilization. The protein part of the allergens mainly contained the fraction with a high molecular weight. Thermal treatment of certain allergens (tularemic and typhoid) was accompanied by the rupture of the bond in the protein molecule, and the separation of fractions with a lesser molecular weight; some retained their specific activity.

The intracutaneous allergic test is broadly employed for the purpose of detecting the immuno-allergic reorganization of the organism caused by vaccination or infection. The appropriate bacterial allergens (tuberculin, tularin, brucellin, anthraxin, pestin, streptococcal, staphylococcal, and whooping cough allergens) are employed in carrying out the test. The methods of preparing

these preparations are extremely diverse. Thus, in preparing tularin the microbial suspension is killed by heating and it is employed at a specific dilution. Brucellin is an unrefined cultural filtrate of a mixture of several types of Brucellae; staphylococcal and streptococcal allergens are fractions isolated from a filtrate of bouillon cultures. A number of allergens are obtained from microbial cells by the employment of various chemical methods. Thus, pestin is a fraction of plague microbes subjected to acid hydrolysis with subsequent fractionation with alcohol. For the preparation of whooping cough allergen the water-ether method of extraction of a microbial suspension is employed.

The allergens employed in practice are complex aggregates of the substances of a bacterial cell or the products of its vital activity and contain various quantities of proteins, nucleic acids, lipids, sugars and low-molecular-nitrogenous compounds. The question of the nature of the substances, which cause allergic skin reactions, has still not been finally resolved, however, in recent times more and more works have been appearing, which attest to the fact that proteins are the carriers of the allergenic properties of the preparations (Seibert, 1955; Morisawa and his coauthors, 1960; Okada, 1963; Tsuverkalov, 1961; Lyanda-Geller, 1963, Mierzejevski, 1966).

The results of the determination of the chemical composition and the biological properties of the fractions obtained by us from different bacteria also give the basis to assume that the allergenic activity of the preparations is connected with the protein part of the cell (Runova and Ulanova, 1967; Gavrilenkova and her coauthors, 1967; Runova and Rudneva, 1968).

By analyzing certain data of the study of the nature of allergens (their thermal stability, their ability to be digested by proteinases, the effect of the reduction in activity upon

iodination, deamination, upon the treating of various kinds of allergens with formaldehyde, the presence of biological activity in low molecular protein fractions), it is possible to make the assumption of the existence of a community of allergenic preparations in the structure (Yamamuta and his coauthors, 1959; Sirks and Bleiker, 1963; Degtyarenko and Rozanov, 1966; Gavrilenkova and her coauthors, 1967; Runova and Andreyeva, 1968). In connection with this we were faced with the problem of developing a single method of preparing allergens from various bacteria with a high content of protein.

The method being proposed by us consists in the extraction of alkali-soluble components from microbial cells dried with acetone, their subsequent precipitation with acid, dialysis and lyophilization. Allergens were obtained from plague, anthracic, tularemia, dysenteric, and typhoid microbes and Escherichia coli by this method.

Tularemia microbes were grown on a solid fish or placental medium with the addition of cysteine. The cultivation of plague, anthracic, and Escherichia coli bacteria was carried out on a solid agar medium prepared on a base of Khottinger meat hydrolysate. The tularemia and plague microbes were grown over a period of 2 days, the Escherichia microbes were grown over a period of 18 hours. The obtained microbial mass was washed with a physiological solution and was dried with acetone. Alkali was added to the dry microbial mass at the rate of 50 ml of a 1% solution of potassium hydroxide per 1 g of bacteria. Extraction was carried out at room temperature over a period of twenty-four hours (it was stirred from time to time). The precipitate was removed by centrifuging over a period of 1 hour at 10,000-12,000 r/min at 5°. The obtained extract was precipitated with 50% acetic acid to 1/4-1/5 of the initial volume. The mixture was held for 2 hours at 5-8°. The settled precipitate was separated by centrifuging for 30 min at 4000-6000 r/min. The precipitate was dissolved

in 1/5 of the initial volume of physiological solution by alkalization to pH 7.2-7.4. The obtained preparation was dialyzed for a period of 18-20 hours with tap water, then for 6 hours with distilled water and then subjected to lyophilization. From the data obtained in determining the chemical indices of the preparations (total nitrogen, protein, sugars, and nucleic acids), it follows, that the method of alkaline extraction ensured the obtaining of preparations with a primary content of protein (60-80%) and with admixtures of other components (4-7% sugar, 1-10% nucleic acids). The different kinds of allergens with respect to chemical composition were close to those in (Table 1). The yield of preparations was 20-30% with respect to the weight of the dry microbial mass.

Table. 1. Characteristics of allergens (average results from 5 series, $M \pm \sigma$).

Allergen	Yield of allergen (in % of dry microbial mass)	Chemical composition (in % of dry substance)				Quantity of dry substance (in μg) in 1 cutaneous dose
		total nitrogen	protein	sugar	nucleic acids	
Anthracic . . .	19,5+0,7	13.8+0,1	79,0+1,4	4,3+0,5	3,8+0,4	100
Plague	29,0+3,5	12,9+0,8	78,5+2,8	6,6+2,3	9,2+0,5	10
Tularemia . . .	20,0+2,7	10,2+1,0	60,3+3,3	6,0+2,1	10,0+0,5	2
Dysenteric	27,0+2,4	12,7+0,5	69,6+3,7	5,6+0,8	0,9+0,2	100
Escherichia coli	24,9+3,8	13,8+0,7	68,7+3,9	7,2+1,3	1,6+0,6	100
Typhoid	21,2±4,1	11,9±0,5	67,4±1,3	7,2±1,7	2,7±0,4	100

The allergenic activity of the preparations was studied on guinea pigs, sensitized to an appropriate culture. For this purpose the animals were immunized with STI (Translator's note: could possibly be Institute of Sanitary Engineering) vaccine (40 million spores), with tularemia vaccine (20 million microbial cells) and with plague vaccine (1 million microbial cells), in accordance with the requirements of the MRTU (Interrepublic Technical Specifications) on the control of allergens; the microbes of the enteric group (dysenteric, typhoid, and Escherichia bacilli) for the purpose of sensitization were introduced subcutaneously in an amount of 10 billion cells of living culture.

A skin test was performed 4 weeks after immunization with vaccines of a group of especially dangerous infections and each 10 days after a single administration of a living culture of microbes of the enteric group. For carrying out the skin tests appropriate dry allergens were dissolved in a borate buffer with a pH of 7.2-7.4 and sterilized by autoclaving for 30 min at 110°.

By titrating various dilutions of allergens their diagnostic doses were established in weight units. The quantity of allergen was accepted as one skin dose, which upon administration to sensitized animals caused a positive skin reaction of the slow type with a diameter of the hyperemia zone of 8-15 mm. For tularemia allergen this dose was 2 μg, for plague allergen - 10 μg, and for the remaining preparations - 100 μg of dry substance. In this case the protein content should be not less than 60% in scaling for the dry weight of the preparation. Considering, that the protein is the active source of the allergens, it is necessary to proportion the quantity of administered preparation in accordance with the protein content.

The obtained data make it possible to recommend a single method for obtaining allergens of bacterial origin. This method is simple and it makes it possible to unify the production of allergens, sufficiently standard with respect to chemical composition and biological (allergenic) properties. In an investigation of the specificity of anthracic, plague, and tularemia allergens it was shown, that each of them caused the appearance of skin reactions only in animals vaccinated with a homologous culture, i.e., the indicated allergens were specific (Table 2).

The allergens of the microbes of the enteric group, as well as commercial dysenterin, caused cross skin reactions in guinea pigs sensitized by the administration of various cultures - dysenteric bacilli and Escherichia coli (Runova and Andreyeva,

1968). This, apparently, attests to the presence in the allergens of the enteric group of general determinant groupings; the presence of these general groups manifests itself in highly sensitized guinea pigs in the form of cross skin reactions. At the same time in people with the employment of allergens of the enteric group (dysenteric bacillus, Escherichia coli) we did not observe analogous phenomena. Thus, those ill with dysentery reacted positively to the dysenteric allergen, in the majority of cases did not react to the introduction of Escherichia coli allergen, which attested to the definite specificity of allergens of the bacteria of the enteric group. However, the question of the specificity of these allergens has still not be sufficiently studied, in connection with which the obtained preparations should be broadly tested under experimental and clinical conditions.

Table 2. The specificity of allergens of the group of especially dangerous infections in tests on pre-inoculated animals.

Animals inoculated with vaccine	Average (of 5 determinations) diameters (in mm) of infiltrates in response to the introduction of allergens (M ± m)			
	plague allergen	anthraxin	tularin	anthacic allergen
Plague	16.2±1,3	—	—	—
Brucellosis	—	—	—	—
Anthracic	—	11±1,7	—	12±1,8
Tularemia	—	—	10±0,9	—
Control (nonimmunized animals)	—	—	—	—

In the previous investigations we studied a number of properties of allergenic preparations: thermal stability, the loss or partial reduction in activity under the effect of proteinases, a number of chemical effects, the possibility of further refining (Gavrilenkova and Pryadkina, 1965; Chernyakhover and his coauthors,

1965; Runova and Ulanova, 1965; Runova and Rudneva, 1968). In this work certain additional physical characteristics of allergens, obtained by the alkaline extraction method, were obtained.

We carried out a tentative determination of the molecular weights of the protein fractions of allergens employing gel filtration based on Sephadex G-200. Both the initial preparations, as well as autoclaved preparations (this method was selected by us for the obtaining of sterile allergens) were subjected to fractionation. The conditions of the experiment were as follows: in a tube (the height was 50 cm and the diameter was 2.5 cm) we placed 6-8 mg of the protein substance; elution from the tube was carried out with a 0.14 M solution of sodium chloride prepared on a phosphate buffer base of 0.001 M with a pH of 7.2. The solution which came out of the tube was collected in a drop collector in fractions with a volume of 5.5 ml and the quantity of protein in them was determined by the Louri (Translator's note: name not verified; could possibly be Lowry) method. The tubes were pre-calibrated with respect to normal rabbit serum. As a result it was demonstrated, that the initial allergens obtained from microbes of especially dangerous infections which had not been subjected to autoclaving contained one protein fraction with a molecular weight of 900,000 (Fig. 1). The allergens isolated from bacteria of the enteric groups also mainly contained a fraction with a molecular weight of 900,000, however for the preparations obtained from dysenteric microbes a fraction in a considerable quantity was detected with a molecular weight of 160,000, and the allergens of Escherichia coli contained a fraction with a molecular weight of 60,000 (Fig. 2).

In certain allergens upon thermal treatment breaking of the bonds occurred with the cleavage of the protein of lesser molecular weight. Thus, the autoclaved tularemia allergen contained in addition a protein fraction with a molecular weight of 160,000 which was present in the preparation in a considerable amount (Fig. 3). In the typhoid allergen after autoclaving

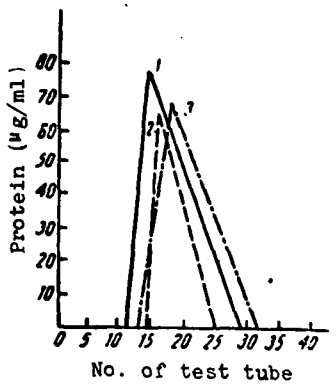

Fig. 1. Gel filtration of nonautoclaved allergens of the group of especially dangerous infections: 1 - tularemia; 2 - anthracic; 3 - plague.

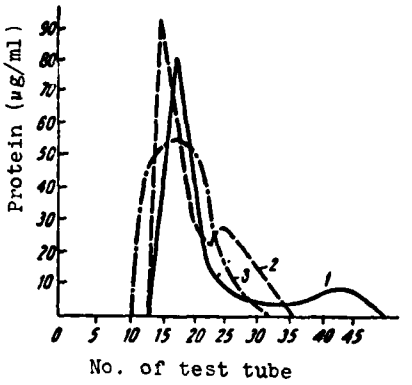

Fig. 2. Gel filtration of nonautoclaved allergens of the enteric group: 1 - Escherichia coli; 2 - dysenteric; 3 - typhoid.

cleavage of the high-molecular protein occurred with the formation of a fraction with a lower molecular weight, as a result the protein fraction with a molecular weight of 160,000 predominated in the autoclaved preparation (Fig. 4).

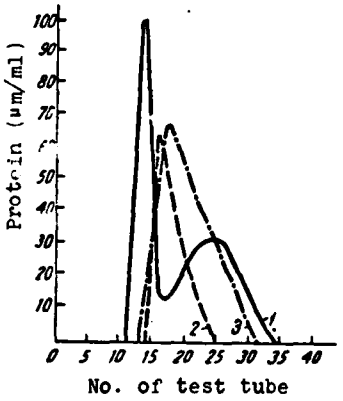

Fig. 3. Gel filtration of autoclaved allergens of the group of especially dangerous infections: 1 - tularemia; 2 - anthracic; 3 - plague.

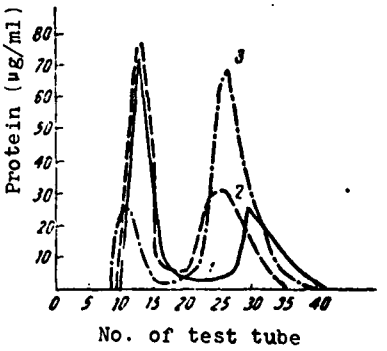

Fig. 4. Gel filtration of autoclaved allergens of the enteric group: 1 - Escherichia coli; 2 - dysenteric; 3 - typhoid.

The allergens obtained from plague, anthracic, and dysenteric microbes and from Escherichia coli varied under the effect of

thermal treatment (see Figs. 3, 4). During biological testing of the fractions both with high (900,000), as well as with relatively low (160,000 and 60,000) molecular weight specific skin reactions were caused in sensitized animals (Table 3) at an appropriate dose (0.5-1 μg of protein for tularemia allergen, 40-60 μg of protein for typhoid and dysenteric allergens).

Table 3. Verification of the biological activity of the fractions with a different molecular weight.

Allergen	Initial		Autoclaved	
	molecular weight	average diameter of the infiltrate (in mm)	molecular weight	average diameter of the infiltrate (in mm)
Tularemia	900,000	20	160,000	18
Typhoid	900,000	8	160,000	9
Dysenteric	900,000	11.5	160,000	12
Escherichia coli	900,000	10	60,000	9

The obtained data once again confirm, that substances of protein nature both with high, and with low molecular weight possess allergenic properties.

Further investigations in this area should be directed at an in-depth study of the active peptides of various microbes for the purpose of decoding the nature of the allergens.

Conclusions

1. A single method is proposed for obtaining allergens from bacteria of a group of especially dangerous infections (anthracic, plague, and tularemia) and from the family of enteric diseases (dysenteric, typhoid bacilli, and Escherichia coli).

2. The alkaline extraction method ensured the obtaining of allergens with a primary content of protein (60-80%) and insignificant admixtures of other components (4-7% sugar and 1-10% nucleic acids).

3. The protein part of the allergens primarily contained a fraction with a molecular weight on the order of 900,000.

4. The heat treating of the preparations led to the breaking of the bond in the protein molecule and to the cleavage of the particle with the lesser molecular weight (160,000 for tularemia and typhoid allergens and 60,000 for Escherichia coli allergen). These fractions possessed specific activity.

BIBLIOGRAPHY

Гавриленкова В. Ю., Прядкина М. Д. В кн.: Материалы межинститутской научной конференции памяти Л. А. Тарасевича. М., 1965, с. 319. — Гавриленкова В. Ю. и др. Там же, 1966, с. 22. — Гавриленкова В. Ю., Рунова В. Ф. Ж. микробиол., 1967, № 5, с. 21. — Лянда-Геллер Б. А. Пробл. туб., 1963, № 9, с. 67. — Рунова В. Ф., Уланова А. А. В кн.: Материалы межинститутской научной конференции памяти Л. А. Тарасевича. М., 1966, с. 228. — Рунова В. Ф., Рудиева О. А. Ж. микробиол, 1968, № 7, с. 139. — Рунова В. Ф., Андреева З. М. Там же, № 9, с. 132. — Рунова В. Ф., Уланова А. А. В кн.: Материалы межинститутской научной конференции памяти Л. А. Тарасевича. М., 1965, с. 323. — Черняховер С. И., Сиротюк Л. В., Рунова В. Ф. Там же, с. 321. — Цуверкалов Д. А. Ж. микробиол., 1967, № 1, с. 85. — Jamamura J., Morisawa S., Tanaka A., et al. Proc. jap. Acad., 1959, v. 35, p. 295. — Morisawa S., Tanaka A., Shojima K. et al. Biochim-biophys. Acta, 1960, v. 38, p. 252. — Seibert F. B., Fugueroa E. L., Dufor E., Am. Rev. Tuberc., 1955, v. 71, p. 704. — Sirks J., Bleiker M., Acta tuberc. scand., 1963, v. 43, p. 87.